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Increased Estrogen Receptor and Epidermal Growth Factor Receptor Gene Product Co-expression in Surgically Resected Gastric Adenocarcinomas

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Background: Evidence exists that estrogens influence the action of epidermal growth factor (EGF) and its receptor (EGF-R) at multiple levels. Estrogen and antiestrogen action on gastric and other gastrointestinal malignancies has been evaluated by several groups with conflicting results, and EGF-R has been implicated in the current growth factor-mediated models for gastric cancer progression.

Methods: ERs and EGF-Rs were detected immunohistochemically in a total of 53 advanced gastric carcinomas using monoclonal antibodies (mAbs) to human ERs and EGF-Rs.

Results: ERs were expressed in 30 (56%) and EGF-Rs in 20 (38%) of the gastric tumors. ER(+) gastric tumors were closely associated with the intestinal type ($P < 0.01$), whereas EGF-R(+) tumors were significantly correlated with poor differentiation status and ER(+) expression ($P < 0.01$). Of EGF-R(+) tumors, 85% were also ER(+). EGF-R and ER co-expression was demonstrated in 17 tumors (32% of the group). These cases were significantly corelated with poor differentiation and large tumor size upon resection ($P < 0.05$).

Conclusions: ER and EGF-R co-expression indicates that a functional interaction between estrogens and EGF may exist in gastric cancer and that when such an interaction becomes operative, it may lead to dedifferentiation and increased tumor growth.

KEY WORDS: steroid receptors, growth factors, autocrine, stomach

INTRODUCTION

Polypeptide growth factors act through their receptors as autocrine and paracrine growth agents in the progression of several tumors [1]. Epidermal growth factor (EGF) and its receptor (EGF-R) seem to play a considerable role in the proliferation and differentiation of mesodermal and ectodermal tissues in normal, inflammatory, and malignant states [2–4]. However, it has been reported that estrogens modulate the autocrine and paracrine actions of the EGF/EGF-R pair by inducing their synthesis, both at a transcriptional and a translational level [5,6].

It has been reported in recent immunohistochemical studies that EGF-R expression in human gastric cancer

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TABLE I. Clinical and Pathological Characteristics of 53 Gastric Cancer Patients

Parameter	No. of patients
Sex, age range, mean age	
Female/male	22/31
Age range	39–78
Mean age	63
Maximum diameter (cm)	
d < 5 cm	15
d > 5 cm	38
Lauren's classification	
intestinal	26
diffuse	18
nonspecified	9
Differentiation	
well and moderate	23
poor	30
Node involvement	
positive	43
negative	10

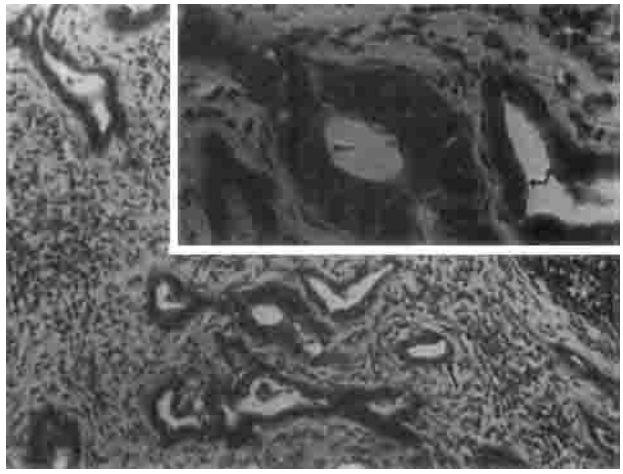

Fig. 1. A moderately differentiated gastric adenocarcinoma demonstrating areas of strong (c, d, +++) cytoplasmic and nuclear staining for estrogen receptors. Arrows indicate stained cells. (×200, Inset; the center of the picture × 400).

correlates with poor differentiation status [7,8]. In addition, we have confirmed previous observations that nuclear and cytosolic expression of estrogen receptors (ERs) is present in human gastric cancer and is associated with high histologic grade [9,10]. Furthermore, the potentially favorable response of some gastric cancer patients to antiestrogen therapy suggests that estrogens and their receptors might contribute in the development of gastric cancer [11].

With special reference to the above, it is possible that the investigation of ER and EGF-R co-expression in gastric cancer might elucidate the functional interaction between estrogens and EGF and their role in the progression of gastric cancer. In the present study, we examined immunohistochemically the ER and EGF-R expression in the postgastrectomy specimens of 53 patients with advanced gastric cancer and correlated the results with several known clinical and pathological parameters.

MATERIALS AND METHODS

Patients and Surgical Specimens

Fifty-three surgically resected gastric cancer samples in formalin-fixed, paraffin-embedded tissue blocks, from patients operated during the years 1991–1992, were obtained from archival material of the First Department of Propedeutic Surgery at Hippocration University Hospital (Athens, Greece). Lymph node status, maximum tumor diameter, patient sex, and mean age were determined. Histomorphological tissue architecture of the tumor samples expressed according to the Lauren's classification scheme and the degree of differentiation were evaluated on hematoxylin-eosin stained sections. All pertinent clincal and pathological data are summarized in Table I.

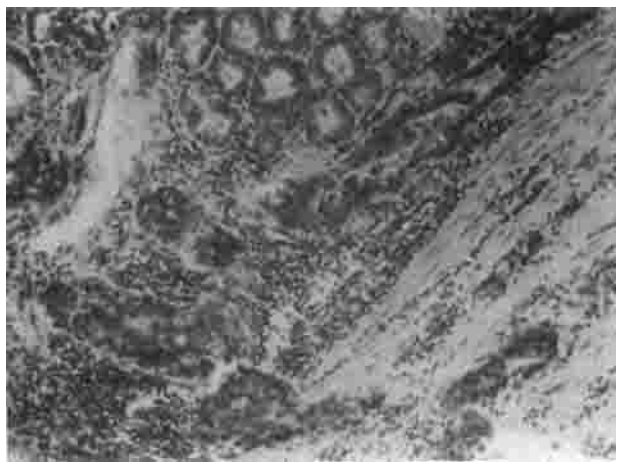

Fig. 2. A moderately (with areas of poorly) differentiated gastric adenocarcinoma and its border with the adjacent benign mucosa, demonstrating strong (c,d,+++) staining for estrogen receptors in the benign component and moderate (c,d,++) staining for estrogen receptors in the neighboring tumor. (×200)

Immunohistochemical Staining

Four 3 mm-thick tissue sections were cut and after deparaffinization through graded alcohols (100%, 90%, 70%), endogenous peroxidase was blocked by incubating the slides in 0.1% hydrogen peroxide of methanol dilution, for 20 min. Immunostaining was performed by the three-step ABC method using the Autoprobe III Rapid immunostaining Kit (Biomeda Corp., Foster City, CA). The primary monoclonal antibodies (mAbs) were used as follows. The ER-ICA monoclonal (Abbot Laboratories, N. Chicago, IL) was used in a prediluted form for 45

TABLE II. Estrogen Receptor (ER), and Epidermal Growth Factor Receptor (EGF-R) Staining vs. Clinicopathological Parameters in 53 Gastric Cancer Patients

	ER expression			EGF-R expression		
Parameter	Present n = 30 (56%)	Absent n = 23 (44%)	*P*-value	Present m = 20 (38%)	Absent m = 33 (62%)	*P*-value
Sex						
Female	13	9		7	15	
Male	17	14	NS	13	18	NS
Maximum diameter						
d < 5cm	9/15	6/15		5/15	10/15	
d > 5cm	21/38	17/38	NS	15/38	23/38	NS
Lauren's classification						
intestinal	19/26	7/26	$P < 0.01$*	12/26	14/26	
diffuse	4/18	14/18		5/18	13/18	
nonclassified	7/9	2/9		3/9	6/9	NS
Differentiation						
well *and*						
moderate	10/23	13/23		4/23	19/23	
poor	20/30	10/30	$P < 0.1$*	16/30	14/30	$P < 0.01$*
Node involvement						
positive	22/43	21/43		17/43	26/43	
negative	8/10	2/10	$P < 0.1$*	3/10	7/10	NS
Estrogen receptors						
positive	—	—		17/30	13/30	
negative				3/23	20/23	$P < 0.01$*

*Statistically significant at $P < 0.05$.
NS = not significant.

min at 37°C, and the EGF-R (Ab-4) (Oncogene Science, Uniondale, NY) in a dilution of 1:50 for 45 min at 37°C. Both mAbs were incubated in a humidified chamber. Tumor slides subjected to the whole procedure, except for the incubation with the primary monoclonal antibody, were used as negative controls, whereas previously known positive endometrial carcinoma and oral squamous cell carcinoma sections were used as positive controls for ER and EGF-R immunoreactivity respectively.

Evaluation of ER and EGF-R Staining

Tumor slides were analyzed and scored according to the ER and EGF-R intensity of staining in positive controls, as 0:negative, + : weak, ++ : moderate, +++ : strong, and according to the percentage of positive cells as a = 0-5% of tumor cells, b = 5-45% of tumor cells, c > 45% of tumor cells. Staining of the neighboring normal or dysplastic mucose was referred to as "d". The interpretation was performed in a double-blinded fashion without synchronous knowledge of the other clinicopathological data. However, for statistical purposes, the data were analysed as simply positive or negative. In addition, tumors with 0–5% positive cells to anyone of the mAbs were not included to the positively stained tumor group.

Statistical Analysis

The Chi-square and Fisher's exact tests were used to determine the level of statistical significance between ER and EGF-R expression and various clinicopathological parameters. A *P* value of equal to or less than 0.05 was considered statistically significant, although insignificant associations ($P < 0.1$) representing clinically important relationships were also mentioned. Relative risk (r,r) was also used to further describe the numerical association of each statistically significant relationship.

RESULTS

Estrogen Receptors (ERs)

Cytosolic and nuclear estrogen receptors (ERs) were expressed in 30 (56%) of the gastric cancer specimens, with ER staining ranging from weak to strong (Fig. 1). Strong (+++) ER staining was also detected in the adjacent normal and dysplastic mucosa of the ER(+) gastric tumors (Fig. 2). When the tumors were classified according to *Lauren's scheme*, ERs were expressed 2.08 times (r.r = 2.08) more frequently in tumors of the intestinal type ($P < 0.01$). Although poorly differentiated tumors tended to express ERs more frequently, this association was insignificant ($P < 0.1$). Tumors with poorly and moderately differentiated cell subpopulations within the same specimen exhibited a more intensified ER expression in areas of poorer differentiation. Furthermore, no significant difference in ER expression was observed with regard to gender, tumor maximal diameter, and node involvement. All assessed data are summarized in Table II.

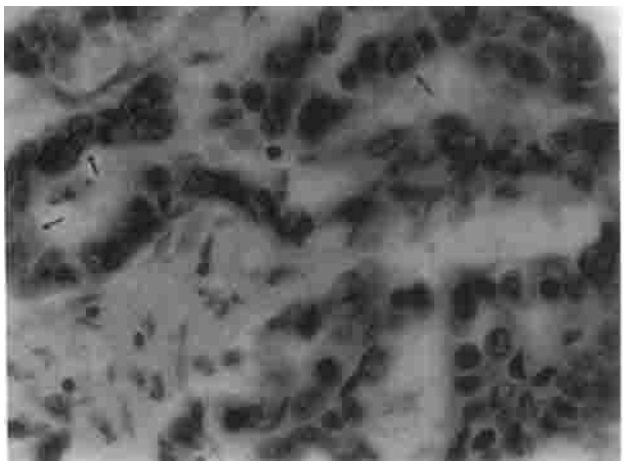

Fig. 3. A case of poorly (with areas of moderately) differentiated gastric cancer demonstrating moderate (c,d,++) cytosolic staining for epidermal growth factor receptors. Arrows indicate stained cells (×400).

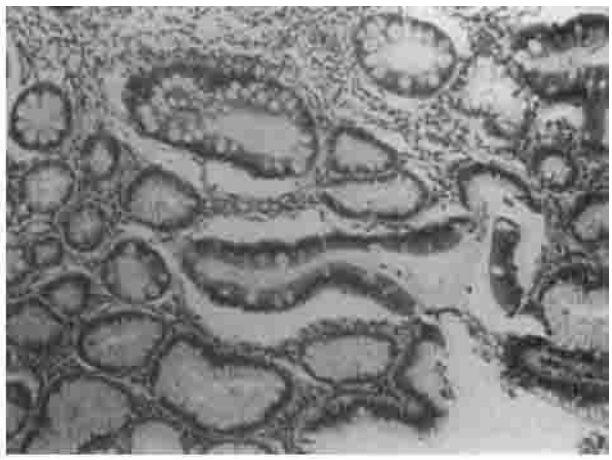

Fig. 4. Areas adjacent to the tumor of the case in Figure 3, with incomplete type intestinal metaplasia demonstrating strong (c,d,+++) cytosolic and membranous staining for epidermal growth factor receptors. (×200)

Epidermal Growth Factor Receptors (EGF-Rs)

Twenty (38%) gastric tumors were positive for EGF-R expression with staining intensity ranging from weak to strong (Fig. 3). The pattern of expression was mainly cytoplasmic, but eight specimens showed membranous staining as well. All EGF-R positive gastric tumors also exhibited EGF-R immunoreactivity in the parietal and chief cells of the gastric glands, in adjacent areas of normal and dysplastic mucosa (Fig. 4). EGF-R was expressed in 16 (53%) of the 30 poorly differentiated tumors, whereas EGF-R (+) staining was detected in 4 (17%) of the 23 well *and* moderately differentiated gastric adenocarcinomas. The difference of EGF-R expression between the two histologic groups was statistically significant ($P < 0.01$). However, EGF-R(+) gastric tumors were not significantly associated with neither the tumors' maximum diameter and *Lauren's class,* nor the nodal involvement (Table II). Surprisingly, tumor infiltrating macrophages and activated plasma cells of one EGF-R(+) gastric tumor expressed strong EGF-R immunoreactivity at such an intensity (+++) that they were used as internal positive controls for the evaluation of staining in adjacent cellular populations (Fig. 5).

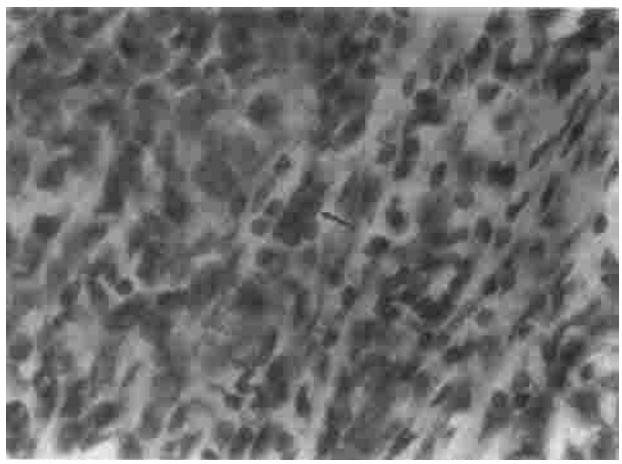

Fig. 5. Strong (c,d,+++) staining for epidermal growth factor receptors, of two stromal macrophages, in a case of gastric cancer which was positive for the above antigen. This strong staining was used as an internal positive control. (×320).

ER and EGF-R Coexpression

The tumor specimens were then classified into the following two groups according to the results of ER and EGF-R status: group 1, with *both* ER and EGF-R presence [ER(+) and EGF-R(+)] and group 2 with either ER or EGF-R presence or absense (i.e., the rest of the group). Synchronous expression of ER and EGF-R was observed in 17 (32%) of the tumors of our group, whereas the remaining 36 patients belonged to group 2. Seventeen (85%) of EGF-R(+) tumors were also ER(+), whereas 56% of ER(+) tumors exhibited EGF-R expression (Table II). Additionally, ER expression in EGF-R (+) gastric tumors was statistically significant ($P < 0.01$).

Table III shows the relationship between the pathological data and tumor ER\EGF-R coexpression. When compared to the rest of the cases, gastric tumors positive to both receptors were significantly correlated with poor differentiation status ($P < 0.025$, r.r = 1.85) and occurred 1.6 times more frequently in larger tumors (max diameter > 5 cm, $P < 0.05$, r.r = 1,6). Insignificant associations were found concerning Lauren's classification and node involvement in relation to ER\EGF-R coexpression.

TABLE III. Estrogen Receptor (ER), and Epidermal Growth Factor Receptor (EGF-R) Co-expression Patterns vs. Clinicopathological Parameters in 53 Gastric Cancer Patients

Parameter	Rest of group $n_1 = 36$	ER(+) and EGF-R(+) $n_3 = 17$	*P* value*
Maximum diameter			
d < 5 cm	13/15	2/15	
d > 5 cm	23/38	15/38	$P < 0.05$
Lauren's classification			
intestinal	16/26	10/26	
diffuse	14/18	4/18	NS
nonspecified	6/9	3/9	
Differentiation			
well *and*		3/23	
moderate	20/23		
poor	16/30	14/30	$P < 0.025$
Node involvement			
positive	28/43	15/43	
negative	8/10	2/10	NS

*Statistically significant at $P < 0.05$.
NS = not significant.

DISCUSSION

In the present study, we examined the mode of ER and EGF-R expression in the primary tumor site of 53 gastric cancer patients. Immunoreactive EGF-Rs were found in 38% of the gastric tumors and their presense was significantly associated with poorly differentiated carcinomas. Our results confirmed previous studies that reported that EGF-Rs are expressed in 30–40% of gastric tumors and are significantly associated with high histological grade [7,12–14]. In accordance with our observations, Sugiyama et al. [13] demonstrated positive EGFR cytoplasmic *and* membranous immunoreactivity of gastric cancer cells. Although EGF-R presense in the adjacent nonmalignant areas was a frequent observation in our study, we could not confirm immunohistochemically a previous study that exhibited an increased EGFR expression in gastric cancer (up to a 320-fold) in comparison with the corresponding normal gastric mucosa [8]. However, a recent study in brain tumors has revealed that EGFR overexpression may not represent storage of biologically active receptors, but nonfunctional products of EGFR gene alterations [15].

In contrast, we were surprised to find an increased proportion of ER positive gastric tumors (56%) in our study group, whereas in previous reports ER positive staining ranged between 23–27% of gastric tumors [9,16]. In addition, we did not find evidence from previous studies to support our observation that ER expression was associated with intestinal type gastric tumors. Conflicting data evolve from previous reports in regard to the correlation of ER expression and poor differentiation status. Whereas our results confirmed a previous study that reported an insignificant association between ER expression and poorly differentiated gastric tumors [16], Yokozaki et al. [9] revealed a significant relationship between ER expression with high histological grade and poor prognosis in gastric cancer. Our report supports previous studies demonstrating a nuclear *and* cytoplasmic localization of ER immunoreactivity in gastric cancer (Fig. 1) [10,17]. However, other research groups have exhibited only nuclear ER expression [9,16]. Interestingly enough, in accordance with a previous report in 16 cases of gastric cancer, 85% of the EGFR(+) gastric tumors of our group also exhibited ER positive staining [9]. Results of two previous studies in breast cancer demonstrating an inverse relationship between ER and EGFR expression are in contrast to our observations [18,19]. Furthermore, we observed that ER(+)/EGFR(+) gastric tumors were significantly associated with poor differentiation status and large tumor size.

The above data associated that ER and EGFR may have a considerable contribution to the progression of gastric cancer and that when such a contribution becomes operative, poor differentiation and increased local growth are likely to occur. It would be of interest to investigate in the future the survival rates of the ER(+)/EGFR(+) gastric cancer patients.

The considerable ER/EGFR co-expression (32%) suggests that a functional interaction between estrogens and EGF may exist in gastric cancer. Consistent with this hypothesis is the demonstration by Ignar-Trowbridge et al. [5] and Mukku et al. [6] that the action of EGF may modulate ER, and vice versa, and estrogens may upregulate EGF autocrine or paracrine action and EGF-R synthesis, via the ER itself. In conclusion, our results reconfirm in a greater patient sample a previous report about ER/EGF-R co-expression [9] and correlate this co-expression with poor differentiation and large tumor size upon resec-

tion, adding another dimension to the autocrine loop model proposed for gastric cancer. Also taking into account the conflicting data arising from in vitro studies and human trials that involve the study of the effects of estradiol [20] and tamoxifen [11,21,22] in gastric cancer cell lines and gastric cancer patients, it would be very interesting to follow the example of other endocrine manipulated tumors [23] and investigate the combined effects of anti-EGF and anti-estrogen agents in the behavior of ER(+)/EGFR(+) gastric cancer cells in vitro or in animal models.

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